IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael Brigham-Burke, *et al.*	: Art Unit:	To Be Assigned
Serial No.:	To be Assigned	: Examiner:	To Be Assigned
Filed:	Herewith	:	
For:	A METHOD OF IDENTIFYING AGONIST AND ANTAGONISTS FOR TUMOR NECROSIS RELATED RECEPTORS TR1 AND TR2	:	

DIVISIONAL OF:

Applicants:	Michael Brigham-Burke, *et al.*	: Art Unit:	1647
Serial No.:	09/072,993	: Examiner:	D. Romeo
Filed:	May 6, 1998	:	
For:	A METHOD OF IDENTIFYING AGONIST AND ANTAGONISTS FOR TUMOR NECROSIS RELATED RECEPTORS TR1 AND TR2	:	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please delete the paragraph beginning on page 1, line 4, and ending on page 1, line 6, in its entirety, and replace with the following replacement paragraph:

This application is a division of application No. 09/072,993, filed May 6, 1998, which claims the benefit of U.S. Provisional Application No. 60/055,513, filed August 13, 1997, U.S. Provisional Application No. 60/056,980, filed August 26, 1997, and U.S.

Provisional Application No. 60/057,550, filed August 29, 1997, the entire contents of all are incorporated herein by reference.

Please replace the paragraph beginning on page 13, line 27, and ending on page 14, line 7, with the following replacement paragraph:

The putative transmembrane domain of translated TR2 sequence was determined by hydrophobicity using the method of Goldman et al. (1) for identifying nonpolar transbilayer helices. The region upstream of this transmembrane domain, encoding the putative leader peptide and extracellular domain, was chosen for the production of an Fc fusion protein. Primers were designed to PCR the corresponding coding region from the TR2 cDNA with the addition of a BglII site, a Factor Xa protease cleavage site and an Asp718I site at the 3' end. PCR with this primer pair (forward 35-mer 5' cag gaa ttc gca gcc atg gag cct cct gga gac tg 3' (SEQ ID NO: 6), and reverse primer 53-mer 5' cca tac cca ggt acc cct tcc ctc gat aga tct tgc ctt cgt cac cag cca gc 3' (SEQ ID NO: 7)) resulted in one band of the expected size. This was cloned into COSFclink to give the TR2Fclink plasmid. The PCR product was digested with EcoRI and Asp718I and ligated into the COSFclink plasmid (2, 3) to produce TR2Fclink. This vector encodes amino acids 1-192 of TR2, followed by the amino acids RSIEGRGT (SEQ ID NO: 8) for Factor Xa cleavage, followed by residues 226-458 (end) of human IgG1. The IgG1 region also has a mutation of Cys230 to Ala (2).

Please replace the paragraph beginning on page 15, line 6, and ending on page 15, line 12, with the following replacement paragraph:

An expression vector was constructed which contained the tPA (tissue plasminogen activator) signal sequence, an 11 amino acid sequence derived from HIV-1 gp120 glycoprotein, six histidines, the enterokinase proteolytic sequence SDDDDK (SEQ ID NO: 9) followed by residues 85-240 of the coding region of TL4. This construct was transfected into COS and CHO cells and resulted in the secretion of a soluble form of TL4 (sTL4). The protein was purified by passage over a NiNTA column (available commercially) which binds

to the polyhistidine sequence at the amino terminus of the fusion protein. Cleavage of the fusion protein with enterokinase yielded mature TL4.

Please delete pages 17-24 in their entirety, and replace with the attached sequence listing.

IN THE CLAIMS:

Please cancel claims 1-12 and add the following new claims:

13. A method for assessing the ability of a candidate compound to compete with the binding of the polypeptide set forth in SEQ ID NO: 3 to the polypeptide set forth in SEQ ID NO: 1 comprising:

(a) contacting a candidate compound with the polypeptide set forth in SEQ ID NO: 1 in the presence of labeled or unlabeled polypeptide as set forth in SEQ ID NO: 3; and

(b) assessing the ability of said candidate compound to compete with the binding of the polypeptide set forth in SEQ ID NO: 3 to the polypeptide set forth in SEQ ID NO: 1.

14. The method of claim 13 in which the polypeptide set forth in SEQ ID NO: 1 is on the surface of a host cell, on a cell membrane or on a solid support.

15. A method for assessing the ability of a candidate compound to compete with the binding of the polypeptide set forth in SEQ ID NO: 1 to the polypeptide set forth in SEQ ID NO: 3 comprising:

(a) contacting a candidate compound with the polypeptide set forth in SEQ ID NO: 3 in the presence of labeled or unlabeled the polypeptide set forth in SEQ ID NO: 1; and

(b) assessing the ability of said candidate compound to compete with the binding of the polypeptide set forth in SEQ ID NO: 1 to the polypeptide set forth in SEQ ID NO: 3.

16. The method of claim 15 in which the polypeptide set forth in SEQ ID NO: 3 is on the surface of a host cell, on a cell membrane or on a solid support.

Respectfully Submitted,

RATNER & PRESTIA

Robert L. Andersen, Reg. No. 25,771
H. Steve Ngo, Reg. No. 42,932
Attorneys for Applicants

RLA/fp
Enclosures: Version with markings to show changes made

Dated: October 25, 2001

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The Assistant Commissioner for Patents is hereby authorized to charge payment to Deposit Account No. **19-2387** of any fees associated with this communication.	**EXPRESS MAIL Mailing Label Number: EL 743 542 246 US** **Date of Deposit: October 25, 2001** I hereby certify that this paper and fee are being deposited, under 37 C.F.R. § 1.10 and with sufficient postage, using the "Express Mail Post Office to Addressee" service of the United States Postal Service on the date indicated above and that the deposit is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231. **KATHLEEN LIBBY**

VERSION WITH MARKINGS TO SHOW CHANGES MADE

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